Annika Kihlander
Hon kommer absolut helst
Fax 59 88 73 00

ELSEVIER

Recombinant Galα1,3Gal-Substituted Mucin/Immunoglobulin Chimeras: A Superior Absorber of Anti-Pig Antibodies

J. Liu and J. Holgersson

PREVIOUSLY, we have described the construction and production of a recombinant mucin (*P*-selectin glycoprotein ligand-1)/immunoglobulin fusion protein (patent pending) that became heavily substituted with Galα1,3Gal epitopes following co-expression with the porcine α1,3 galactosyltransferase in COS cells. We could show that approximately 300 ng of PSGL1/mIgG$_{2b}$ immobilized on agarose beads could remove all pig endothelial cell cytotoxic antibodies from 1 m of pooled human AB serum.

Human AB serum was absorbed on agarose beads carrying PSGL1/mIgG$_{2b}$ or pig thyroglobulin (Sigma), or on Galα1,3Gal covalently linked via a flexible poly[*N*-(2-hydroxyethyl)acrylamide] spacer to macroporous glass beads (MPG; Synthesome). Pig endothelial cell cytotoxicity of absorbed and nonabsorbed human AB serum was assessed in ^{51}Cr release assays. The number of Galα1,3Gal epitopes carried on PSGL1/mIgG$_{2b}$ was estimated based on the staining intensity of *Bandereia simplicifolia* IB$_4$ in Western blots using pig thyroglobulin as reference.

Two hundred microliters of agarose beads carrying ≈200 ng (≈1 pmol/L) of PSGL1/mIgG$_{2b}$ removed more pig endothelial cell cytotoxic antibodies from 1 mL of pooled human AB serum than did 200 μL of agarose beads carrying ≈800 μg (≈1 nmol/L) of pig thyroglobulin and 100 mg (500 μL) of Galα1,3Gal-PAA-MPG beads carrying ≈85 μg (≈250 nmol/L) of Galα1,3Gal. PSGL1/mIgG$_{2b}$ had six times more BSA IB$_4$-reactive epitopes than pig thyroglobulin based on densitometric analysis of BSA IB$_4$-stained Western blots.

Finally, PSGL1/mIgG$_{2b}$ is a very effective absorber of anti-pig antibodies—an effectiveness that cannot solely be explained by the higher number of Galα1,3Gal epitopes expressed on this protein. Instead, additional structural features of the protein and its oligosaccharide side chains must contribute to its effectiveness.

From Huddinge University Hospital, Huddinge, Sweden.

Address reprint requests to Jining Liu, Division of Clinical Immunology, F79, Karolinska Institutet, Huddinge University Hospital, SE-141 86 Huddinge, Sweden.

0041-1345/00/$–see front matter
PII S0041-1345(00)01010-1